Environmental and Experimental Botany 147 (2018) 138–146

Contents lists available at ScienceDirect

Environmental and Experimental Botany

journal homepage: www.elsevier.com/locate/envexpbot

Impact of heat-wave at high and low VPD on photosynthetic components of wheat and their recovery

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ARTICLE INFO

Keywords:
Biochemical limitation
Climate change
Diffusional limitation
Heat stress
Photosystem II
Rubisco carboxylation efficiency
Vapor pressure deficit

ABSTRACT

Indirect effects of high temperature through increased vapor pressure deficit (VPD) are vital but often ignored in climate impact studies. We investigated the direct (*via* heat) and indirect (*via* VPD) effects of a post-anthesis applied high temperature episode on biochemical and diffusional components of photosynthesis in two wheat cultivars. Plants were exposed to a five-day episode of hot humid (HH: 36 °C; 1.96 kPa) or hot dry (HD: 36 °C; 3.92 kPa) climate, including normal climate (NC: 24 °C; 1.49 kPa VPD) as control. The capacity and sensitivity of different photosynthetic components that included diffusional and biochemical processes was investigated both during the stress and after six-days of recovery under NC. The results showed that, regardless of VPD, photosynthetic capacity under high temperature was largely impaired by biochemical limitations while the diffusional limitations were relatively insignificant. The processes involved in CO_2-use (*i.e. in vivo* carboxylation efficiency and V_{cmax}) presented higher sensitivity than the processes involved in light-use (PSII efficiency, quantum yield and chlorophyll content index). Maximum photosynthetic capacity under high temperature was primarily restricted by limitations to RuBP-regeneration. Effects of higher VPD through diffusional limitations (stomatal and mesophyll conductance) were potentially insignificant. The effects of VPD through changes in transpiration were observed under HH, where significantly higher g_s and increase in g_s (and transpiration) over time due to acclimation led to drought, because the irrigation amounts per day remained same over the stress period. Resultantly, the photosynthesis under HH was co-limited by both RuBP-carboxylation and RuBP-regeneration. Irrespective of VPD, the after-effects of heat-wave were either non-existent or potentially insignificant, as most of the photosynthetic and related parameters recovered to their respective control. The results indicated that the effect of VPD through changes in soil moisture dynamics is an important confounding variable to consider in climate-impact studies. Higher sensitivity of CO_2-use suggested that even moderately high temperature-episodes might limit photosynthetic capacity and hence crop productivity, thus reiterating the need to develop crop cultivars with greater tolerance to high temperatures.

1. Introduction

With the increase in atmospheric CO_2 concentration, global mean air temperature is expected to rise on average 0.3–0.6 °C per decade, with heat waves increasing in frequency, intensity and duration (IPCC, 2013) enlarging the area under threat of increased climatic variability (IPCC, 2012; Trnka et al., 2014). These conditions are expected to challenge crop production and food security for the growing global

Abbreviations: A_{sat}, maximum net CO_2 assimilation rate at saturating light; A-C_i curve, photosynthetic CO_2 response curve; A_{max}, maximum net photosynthetic rate at saturating- light and CO_2; A-Q curve, photosynthetic light response curve; *AQY*, *apparent quantum yield*; CE, *in vivo* rubisco carboxylation efficiency; C_i, intercellular CO_2 concentration; F_v/F_m, maximum efficiency of photosystem II; g_m, mesophyll conductance; g_s, stomatal conductance; HD, hot dry; HH, hot humid; *J*, rate of electron transport for RuBP regeneration; LCP, light compensation point; l_s, stomatal limitation to photosynthesis; NC, normal climate; PAR, photosynthetically active radiation; PSII, photosystem II; R_d, day respiration; R_{dark}, dark respiration; SPAD, chlorophyll content index; Γ, CO_2 compensation point; TR, transpiration; V_{cmax}, maximum carboxylation rate by Rubisco; VPD, vapor pressure deficit

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https://doi.org/10.1016/j.envexpbot.2017.12.009
Received 15 October 2017; Received in revised form 6 December 2017; Accepted 8 December 2017
Available online 15 December 2017
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population, and thus merit studying the capacity of major staple-food crops (*e.g.* wheat) to tolerate, respond to, and recover from, extreme climatic conditions, including heat waves.

Wheat, being very sensitive to high temperature at the anthesis- and grain-filling stages (Wollenweber et al., 2003) has already been reported to be suffering in terms of lower grain yield (Asseng et al., 2015; Asseng et al., 2011; Ferris et al., 1998; Porter and Gawith, 1999). Since the reduction in crop yield under heat stress is also the result of lower photosynthetic carbon-use (assimilation) and higher carbon-loss (respiration) (Ainsworth and Ort, 2010; Atkin et al., 2006), the direct and indirect effects of high temperature on photosynthetic capacity and its ability to recover after alleviation of stress periods need to be explored.

There are a number of ways by which high-temperatures can directly limit the CO_2 assimilation in C_3 plants, *i.e.* decreased ratio between the solubility of CO_2 and O_2, increased photorespiration, reduced activity of Rubisco (Farooq et al., 2011), and direct damage to photosynthetic apparatus (PSII) under extreme high temperatures (> 45 °C) (Sharkey, 2005). Whether light dependent processes (light-use) or/and dark reactions (CO_2-use) are more sensitive to heat stress remains unclear because of contrasting evidences in the literature. Some reports (Law and Crafts-Brandner, 1999; Salvucci and Crafts-Brandner, 2004; Salvucci et al., 2001) have indicated that Rubisco-activation is the primary constraint in decreasing CO_2 assimilation under moderate heat stress because Rubisco activase is very heat labile (Salvucci et al., 2001). Plants can tolerate heat by maintaining a higher Rubisco activation state (Stasik and Jones, 2007), because the Rubisco enzyme itself is very stable at high temperatures (at least 50 °C) (Crafts-Brandner and Salvucci, 2000; Eckardt and Portis, 1997; Sage et al., 2008) and its activity increases with temperature (Salvucci and Crafts-Brandner, 2004). However, direct damage to Rubisco is also possible at higher temperatures (Medlyn et al., 2002).

Photosynthetic light-dependent processes have been shown to be sensitive to heat stress (Allakhverdiev et al., 2008; Yamane et al., 1998). Yet, some studies have indicated that PSII activity remains unaffected even at temperatures that can inhibit CO_2 fixation, suggesting it to be less sensitive to moderate high temperatures (usually < 40 °C) (Sharkey, 2005; Stasik and Jones, 2007). Moderately high temperatures, however, has been reported to increase the alternate electron flow (PSI cyclic electron flow) (Zhang and Sharkey, 2009), which can also limit photosynthesis (Feller et al., 1998).

Furthermore, high temperatures can have indirect effects on photosynthetic carbon-use. Temperature induced increase in vapor pressure deficit (VPD) increases plant evapotranspiration (ET), and if the soil water is limited, higher ET may lead to drought (Will et al., 2013), thus creating an additional stress. However, the increase in transpiration (TR) also depends on stomatal conductance (g_s) which, depending on cultivars/species, may have either a linear or curvilinear response to increasing VPD (Kröber and Bruelheide, 2014; Kröber et al., 2015). Lower g_s at high VPD can limit TR and could also limit photosynthesis due to lower intercellular CO_2 concentration (C_i) (Perez-Martin et al., 2009). On the other hand, g_s has been reported to increase exponentially with temperature (Bunce, 2000), thus higher g_s even at relatively lower VPD may lead to higher TR. Hence, whether or not changes in g_s affects photosynthetic rate depends on changes in C_i and TR.

A literature survey indicated that most of the photosynthetic studies pertaining to heat effects have either focused upon CO_2-use (Calvin cycle processes) or light-use (PSII efficiency and activity). Hence, a comprehensive study on the effects (and indeed after-effects) of heat wave on both CO_2 and light-use would be helpful in understanding the sensitivity of the whole photosynthetic process. Moreover, the indirect effect of high-temperature through increased VPD on diffusional and metabolic photosynthetic limitations is little known. We tested these hypotheses: (i) Heat wave will reduce the photosynthetic capacity of wheat mainly by affecting the processes involved in CO_2-use; (ii) Effects of heat-wave on photosynthesis will exacerbate at high VPD due to increased diffusional and metabolic limitations; (iii) As heat stress can permanently damage the photosynthetic apparatus, we further hypothesise that the effect of heat wave will be long-lasting without full recovery after alleviation of the stress. Therefore, the objectives of this work were threefold: (i) to examine the effects of post-anthesis heat wave with high and low VPD on photosynthetic components relating to diffusional (stomatal) and metabolic limitations in two wheat cultivars; (ii) exploring whether light- or CO_2-use is more sensitive under moderately high temperature; (iii) to study if the effects of heat wave and VPD on photosynthetic parameters are chronic or short-lived with the possibility to recover.

2. Materials and methods

2.1. Experimental setup

An experiment was carried out in climate chambers (see below for details) from October 2015 to February 2016 at the Research Centre Foulum, Aarhus University, Denmark. Seeds of two wheat cultivars (Taifun and Vinjett) which showed differences under heat stress in previous studies (Vignjevic et al., 2015; Wang et al., 2015) were sown in ten litre volume plastic pots filled with mixture (9 kg, 1:1 v/v) of sandy soil (sand 68%, clay 8%, silt 21%, humus 2.7%; Jyndevad, Denmark) and peat (substrate no. 4, size 10–30 mm, pH 5.6–6.4: Pindstrup Mosebrug A/S, Denmark). Pots were kept in a greenhouse at sowing (13 October), and later on at three leaf stage (36 days after planting; hereafter DAP) transferred to the climate chambers where typical Danish growing season climate (April to August) for spring wheat was maintained. The daily mean temperature ranged from 10 to 18 °C, relative humidity 70–80%, day length 10–16 h with light intensity of 1100 μmol $m^{-2} s^{-1}$ and CO_2 concentration of 400 ppm. Before transferring the pots to climate chambers (34 DAP), thinning was done to achieve 20 plants per pot. Pot weight at field capacity was estimated by saturating the pots with water, followed by free drainage for three days with top of the pots covered with plastic sheets. Plants were kept well-watered to full pot water-holding capacity throughout the experiment.

Six days after the mid-flowering stage (GS-65, BBCH scale) (Lancashire et al., 1991), plants were exposed to three different climates in separate growth chambers (climate treatments; Table 1). The growth chambers (2.40 × 1.97 × 2.05 m width, length, height) were identical with precise control of light intensity, temperature, CO_2 concentration and relative humidity (MB-Teknik, Brøndby, Denmark). Light intensity (PAR) was set at 1000 μmol $m^{-2} s^{-1}$ during the day. Day and night durations were 16 and 8 h, respectively. PAR, relative humidity and temperature were increased and decreased in steps during the dawn and dusk, but these variables were kept unchanged from 9am to 5pm at the levels shown in Table 1. Climate treatments *i.e.* normal (NC), hot humid (HH) and hot dry (HD) lasted for 5 days, and after that plants were returned to normal climate for recovery. During the treatment period 1000, 1500 and 2000 ml water was applied every morning to pots under NC, HH and HD treatments, respectively. Water was applied through a temporary drip irrigation system which ensured

Table 1
Climatic conditions set for different treatments in the climate chambers.

Chamber	Treatment	Time	Temp (°C)	RH (%)	VPD (kPa)
I	Normal climate (NC)	day	24	50	1.49
		night	12	76	0.34
II	Hot humid climate (HH)	day	36	67	1.96
		night	26	90	0.34
III	Hot dry climate (HD)	day	36	34	3.92
		night	26	80	0.67

Temp, temperature; RH, relative humidity; VPD, vapor pressure deficit; NC, HH and HD are the notations for different climate treatments in text. CO_2 was maintained at 400 ppm.

uniform soil moisture supply on temporal (throughout the day) and spatial (within the pot) scales during the stress period. Plants were fertilized 23 and 40 DAP with inorganic liquid fertilizer (Prima vækst-thusgødning, Denmark). NPK was applied on pot surface area basis at the rate of 200:34:158 kg NPK ha^{-1}. Other nutrients included Cu (0.005%), Mg (0.01% + additional $MgSO_4$), B (0.01%), Mo (0.001%), Zn (0.0025%) and Mn (0.01%). Plants were sprayed with flexity (metrafenone) against powdery mildew before the start of heading (48 DAP). The experiment included 24 pots (3 climates × 2 cultivars × 4 replicates).

2.2. Measurements and calculations

Chlorophyll content index (SPAD) was measured from the centre of the flag leaves (n = 3) using SPAD meter (Hydro, Japan) on the fifth day of stress (stress day; SD hereafter) and six days after the stress. Chlorophyll fluorescence parameter F_v/F_m (maximum quantum efficiency of photosystem II) was also measured (n = 3) on the same dates as SPAD using the Mini-PAM fluorometer (Walz, Germany) following Genty et al. (1989).

Photosynthetic light response curves (*A-Q* curves) were generated during the stress period (SD4 and SD5) and 6 days after the stress using the CIRAS-2 portable photosynthesis system (PP systems Inc. MA, USA). Flag leaves (n = 3) were first acclimatized to highest PAR level (1500 μmol $m^{-2} s^{-1}$) and then PAR was reduced to 20 μmol $m^{-2} s^{-1}$ in ten steps (*i.e.* 1500, 1100, 800, 550, 400, 300, 200, 100, 60 and 20). Photosynthesis for each irradiance level was recorded after values had stabilized, usually after two minutes. The cuvette environment (temperature and CO_2) was set to match the ambient conditions during all measurements. The cuvette VPD during the measurements under NC, HH and HD climates was 1.35, 1.56 and 3.33 kPa, respectively. Flow rate in the cuvette was 250 μmol $m^{-2} s^{-1}$. Measurements after the stress period were done under NC.

Light saturated photosynthetic response curves (n = 3) to increasing levels of CO_2 concentrations (*A-C_i* curves) were generated on the same dates as *A-Q* curves using a second unit of CIRAS-2. *A-C_i* curves were generated by increasing CO_2 levels from 20 to 1500 μmol $m^{-2} s^{-1}$ in ten steps (*i.e.* 20, 60, 100, 200, 300, 400, 500, 800, 1100, and 1500). The measurements were taken after photosynthesis had stabilized. The PAR in the cuvette was 1500 μmol $m^{-2} s^{-1}$ while the temperature was set to match the ambient conditions. The cuvette VPD under NC, HH and HD climates was 1.39, 1.65 and 3.42 kPa, respectively. Measurements after the stress period were done under NC.

Photosynthetic parameters *i.e.* dark respiration (R_{dark}), light compensation point *(LCP),* apparent quantum yield *(AQY)* and A_{sat} (maximum net CO_2 assimilation rate at 400 ppm external CO_2 and saturating light) were estimated by fitting *A-Q* curves (Fig. 1) to the non-rectangular hyperbola model of Marshall and Biscoe (1980) using the utility tool "Light Response Curve Fitting 1.0" available at http://landflux.org/Tools.php. This tool is based on GRG nonlinear least squares curve fitting procedure in Microsoft excel.

Photosynthetic parameters and limitations, *i.e.* V_{cmax} (maximum rate of carboxylation by Rubisco), *J* (electron transport demand for RuBP regeneration), R_d (day respiration), chloroplast CO_2 compensation point (Γ), mesophyll conductance (g_m) and A_{max} (maximum photosynthetic capacity; net photosynthesis at 1500 μmol $m^{-2} s^{-1}$ irradiance and 1500 ppm external CO_2), were estimated by fitting the *A-C_i* curves (Fig. 2) to the photosynthesis model for C_3 plants by Farquhar et al. (1980) as described by Ethier and Livingston (2004). Curves were fitted using a Microsoft excel utility tool "A/Ci Response Curve Fitting 10.0" available at http://landflux.org/Tools.php. Ratio of *J* to V_{cmax} was calculated. For comparison, parameters V_{cmax}, *J*, R_d and g_m were normalized to 25 °C using the equations from Harley et al. (1992) as described by Sharkey et al. (2007). Temperature response functions used for normalizing the parameters were from Bernacchi et al. (2003, 2001). Parameters normalized to 25 °C are denoted as $V_{cmax.25}$, $J_{.25}$, $R_{d.25}$ and $g_{m.25}$. The relative stomatal limitation to photosynthesis (l_s) was calculated from *A-C_i* curves by the method of Farquhar and Sharkey (1982), and the values are presented in percentage. In this method, g_m is considered to be a non-limiting factor to photosynthesis which can be true for wheat (Perdomo et al., 2017). The actual carboxylation efficiency *in vivo (CE)* was estimated by linear regression from the initial slope of *A-C_i* curves (Parsons et al., 1998; Stasik and Jones, 2007).

2.3. Statistics

Data were checked for normality (Shapiro and Wilk, 1965) followed by log transformation if needed to obtain variance homogeneity. The effects of climate, cultivar and their combination on different parameters were analysed using two-way ANOVA with Statistix ver. 8.1 (Statistix, Tallahassee, FL, USA). Multiple comparisons of treatment means were performed using Tukey's HSD test at 5% level of significance. Correlation analysis for some of the parameters was also performed using the same software.

3. Results

3.1. Stress period

3.1.1. Apparent quantum yield (AQY), dark respiration (R_{dark}), light compensation point (LCP) and net assimilation rate at saturating light (A_{sat})

The apparent quantum yield during the stress period was significantly affected by climate and cultivar (Tables 2 and 3). Taifun cultivar had higher *AQY* than Vinjett. HD climate significantly reduced *AQY* compared to NC climate. R_{dark} was significantly higher in HH and HD climates compared to NC (Tables 2 and 3). Likewise, *LCP* was significantly higher under HH and HD when compared to NC climate. A_{sat} was not affected by any of the treatment or treatment combinations (Tables 2 and 3).

3.1.2. Maximum carboxylation rate by Rubisco ($V_{cmax.25}$), rate of electron transport for RuBP regeneration ($J_{.25}$), $J_{.25}$ to $V_{cmax.25}$ ratio, carboxylation efficiency (CE) and net photosynthesis at saturating light and CO_2 (A_{max})

The maximum carboxylation rate by Rubisco ($V_{cmax.25}$), $J_{.25}$ and A_{max} were all affected by climate, while $J_{.25}$ and A_{max} were also affected by climate × cultivar (Table 2). $V_{cmax.25}$ and $J_{.25}$ were significantly reduced under HH and HD when compared to NC climate (Table 4). Significant differences between HH and HD were noticed for $J_{.25}$, which was higher under HD. The two cultivars differed significantly for $J_{.25}$, but only under NC where it was higher for Taifun. The ratio of $J_{.25}$ to $V_{cmax.25}$ was also significantly reduced under HH and HD compared to NC, but with no significant difference between the two hot climates (Tables 2 and 4). A_{max} was significantly higher under HD than for NC and HH. Cultivars differed for A_{max} but only under NC (Table 4). The changes in the absolute values (without normalization; Supplementary material Table 1S) of V_{cmax} and *J* correlated well with the corresponding modelled values of maximum CO_2 assimilation rate (A_{max}) (Fig. 3). However, the correlation between V_{cmax} and *J*, and V_{cmax} and A_{max} at different growth temperatures (24 and 36 °C) was not conserved. The correlation between V_{cmax} and *J* ($R^2 = 0.51$, $P > 0.05$) and V_{cmax} and A_{max} ($R^2 = 0.46$; $P < 0.01$) at 24 °C was weaker than at 36 °C ($R^2 = 0.82$; $P < 0.001$ and $R^2 = 0.80$; $P < 0.001$, respectively). *In vivo* carboxylation efficiency of Rubisco *(CE)* was significantly lower under HH compared to NC and HD (Tables 2 and 4).

3.1.3. Day respiration ($R_{d.25}$), mesophyll conductance ($g_{m.25}$), CO_2 compensation point (Γ) and relative stomatal limitation to photosynthesis (l_s)

Dark respiration ($R_{d.25}$), $g_{m.25}$, Γ and l_s were all affected by climate (Table 2). $R_{d.25}$ and $g_{m.25}$ were significantly reduced under HH and HD when compared to NC (Table 4). Γ was significantly increased under

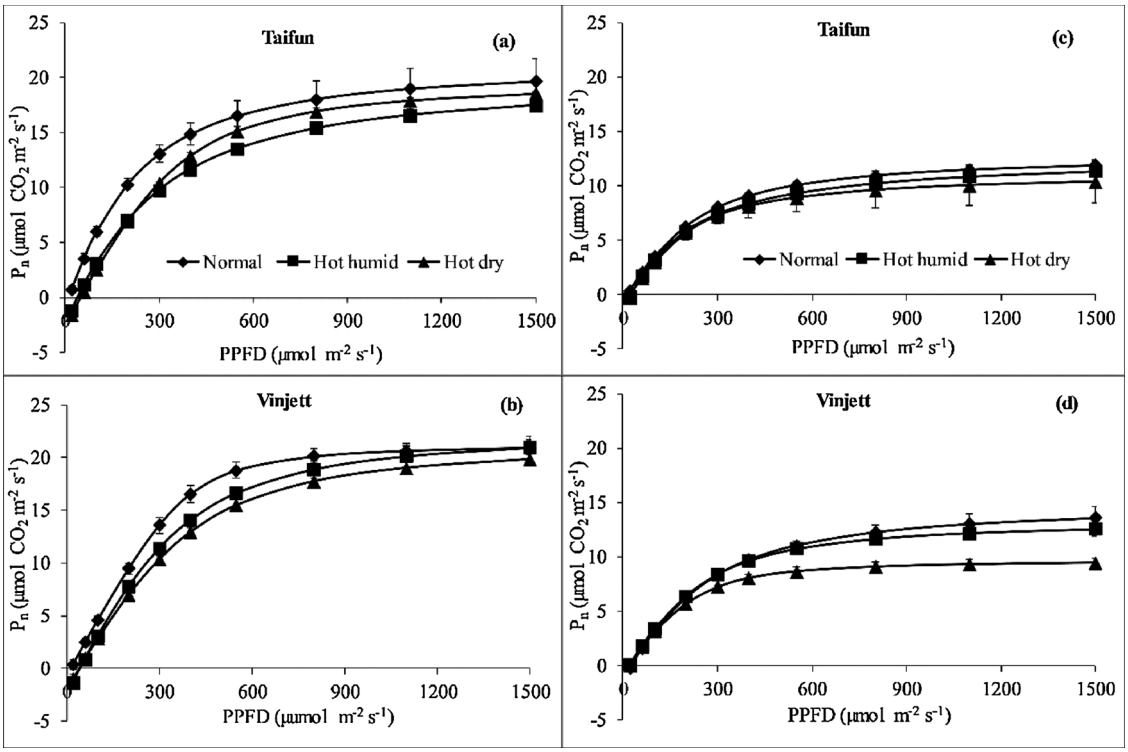

Fig. 1. Photosynthetic light response *(A-Q)* curves of wheat cultivars (Taifun and Vinjett) as affected by climate (normal: 24 °C, 1.49 kPa VPD; hot humid: 36 °C, 1.96 kPa VPD; and hot dry: 36 °C, 3.92 kPa VPD) during stress (a, b) and after stress (c, d), error bars represent ± SE (n = 3).

HH and HD compared to NC, with the highest value under HH climate (Table 4). Stomatal limitation *(l_s)* was significantly higher under NC and HD compared to HH climate (Table 4).

3.1.4. Maximum quantum efficiency of photosystem II (F_v/F_m) and chlorophyll content index (SPAD)

Maximum efficiency of PSII (F_v/F_m) was not affected by any of the treatment factors (Table 2; Fig. 4). SPAD was significantly higher for Taifun cultivar than Vinjett (Table 2; Fig. 5).

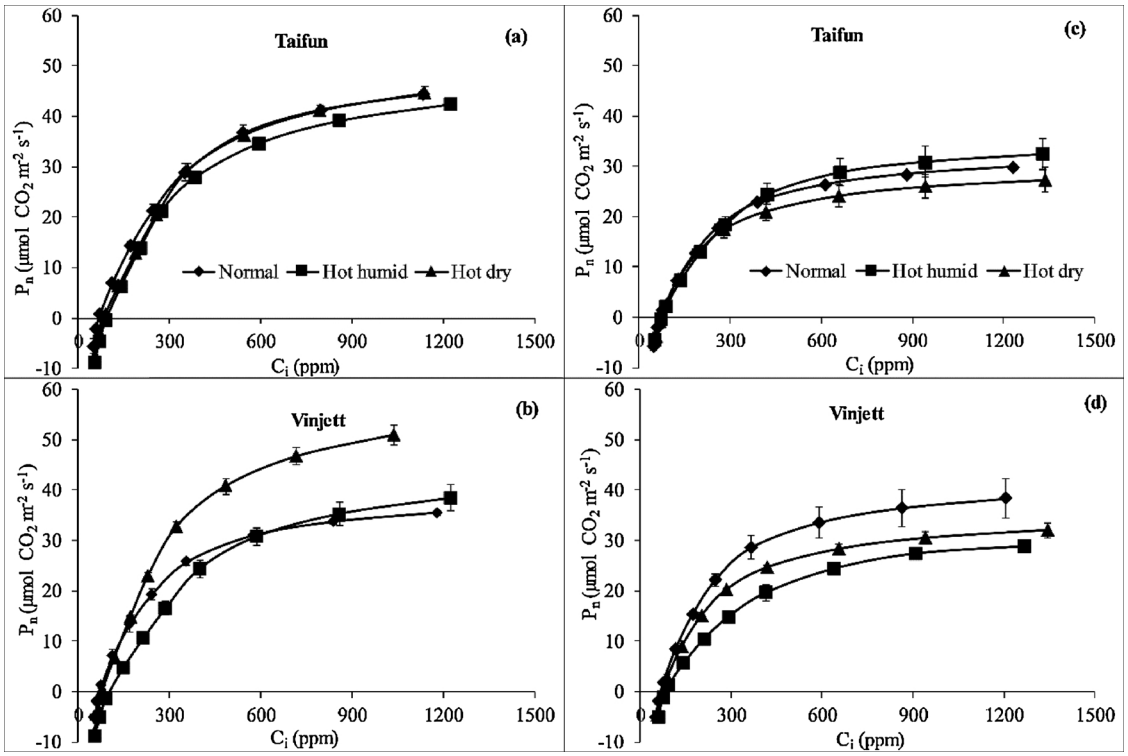

Fig. 2. Photosynthetic CO_2 response *(A-C_i)* curves of wheat cultivars (Taifun and Vinjett) as affected by climate (normal: 24 °C, 1.49 kPa VPD; hot humid: 36 °C, 1.96 kPa VPD; and hot dry: 36 °C, 3.92 kPa VPD) during stress (a, b) and after stress (c, d). C_i is the intercellular CO_2 concentration, error bars represent ± SE (n = 3).

Table 2
Analysis of variance for different plant parameters measured during and after the stress period.

Stage	Treatment	Parameters from A-C_i curves									Parameters from A-Q curves				Additional data	
		$V_{cmax.25}$	$J_{.25}$	$J_{.25}/V_{cmax.25}$	$R_{d.25}$	$g_{m.25}$	Γ	A_{max}	CE	l_s	A_{sat}	AQY	LCP	R_{dark}	F_v/F_m	$SPAD$
Stress	climate	*	***	*	**	***	***	***	*	***	ns	*	***	**	ns	ns
	cultivar	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	*	ns	ns	ns	**
	climate × cultivar	ns	*	ns	ns	ns	ns	**	*	*	ns	ns	ns	ns	ns	ns
Post-stress	climate	*	ns	*	ns	ns	ns	ns	*	**	ns	ns	ns	ns	***	ns
	cultivar	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	**
	climate × cultivar	ns	*	ns	ns	ns	ns	*	ns	ns	ns	ns	ns	ns	ns	ns

Anova table showing the level of significance for the maximum rate of carboxylation by rubisco ($V_{cmax.25}$), electron transport rate for RuBP regeneration ($J_{.25}$), ratio of $J_{.25}$ to $V_{cmax.25}$, day respiration (R_d), CO_2 compensation point (Γ), mesophyll conductance ($g_{m.25}$), net photosynthesis at 1500 ppm of CO_2 and 1500 µmol m^{-2} s^{-1} PAR (A_{max}), *in vivo* carboxylation efficiency of Rubisco (CE), maximum net photosynthesis at saturating light and 400 ppm CO_2 (A_{sat}), Apparent quantum yield (AQY), light compensation point (LCP), dark respiration (R_{dark}), maximum efficiency of photosystem II (F_v/F_m), chlorophyll content index (SPAD) and stomatal limitation to photosynthesis (l_s) as affected by climate, cultivar and climate × cultivar during stress and after stress. '*', '**' '***' indicate significance levels at $p \leq 0.05$, $p \leq 0.01$, and $p \leq 0.001$ respectively, ns is non-significant. A-C_i and A-Q are photosynthetic CO_2 and light response curves, respectively.

Table 3
Parameters estimated from photosynthetic light-response curves during the stress period.

Climate	Taifun	Vinjett	Mean	Taifun	Vinjett	Mean
	AQY (mol mol^{-1})			A_{sat} (µmol CO_2 m^{-2} s^{-1})		
Normal (NC)	0.085	0.054	0.069 A	22.4	22.3	22.3
Hot humid (HH)	0.066	0.059	0.062 AB	22.6	25.3	23.9
Hot dry (HD)	0.055	0.051	0.053 B	22.7	23.8	23.2
Mean	0.068 A	0.055 B		22.6	23.8	
	R_{dark} (µmol CO_2 m^{-2} s^{-1})			LCP (µmol CO_2 m^{-2} s^{-1})		
Normal (NC)	0.91	0.70	0.81 B	10.60	11.80	11.20 B
Hot humid (HH)	2.44	2.52	2.48 A	39.73	43.19	41.46 A
Hot dry (HD)	2.67	2.03	2.35 A	49.74	40.23	44.98 A
Mean	2.01	1.75		33.36	31.74	

AQY, apparent quantum yield; A_{sat}, maximum net photosynthesis at saturating light and 400 ppm CO_2; R_{dark}, dark respiration; LCP, light compensation point. Values sharing the same capital letter, in a column, and in a row, do not differ significantly at P = 0.05 (Tukey HSD). Values sharing the same lower-case letter for climate-cultivar interaction, do not differ significantly at P = 0.05 (Tukey HSD).

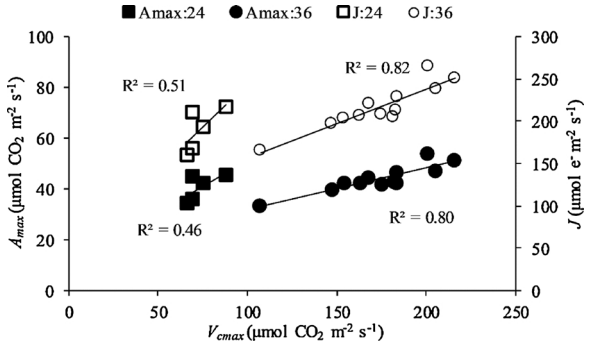

Fig. 3. Correlation among A_{max} (maximum net photosynthesis at 1500 PAR and 1500 ppm CO_2), V_{cmax} (maximum rate of carboxylation by rubisco) and J (electron transport rate for RuBP regeneration) estimated from AC_i curves during the stress period. The numbers "24" and "36" in the legend are the growth and measurement temperatures in °C.

3.2. Post-stress period (after-effects)

3.2.1. Apparent quantum yield (AQY), dark respiration (R_{dark}), light compensation point (LCP) and maximum net assimilation rate at saturating light (A_{sat})

None of these parameters showed statistically significant differences for climate, cultivar and climate × cultivar after the stress period (Tables 2 and 5).

3.2.2. Maximum carboxylation rate by Rubisco ($V_{cmax.25}$), rate of electron transport for RuBP regeneration ($J_{.25}$), $J_{.25}$ to $V_{cmax.25}$ ratio, carboxylation efficiency (CE) and net photosynthesis at saturating light and CO_2 (A_{max})

The maximum carboxylation rate by Rubisco ($V_{cmax.25}$) was only

Table 4
Parameters estimated from photosynthetic CO_2 response curves during the stress period.

Climate	Taifun	Vinjett	Mean	Taifun	Vinjett	Mean	Taifun	Vinjett	Mean
	$V_{cmax.25}$ (µmol CO_2 m^{-2} s^{-1})			$J_{.25}$ (µmol e^- m^{-2} s^{-1})			$J_{.25}/V_{cmax.25}$		
Normal (NC)	85.1	94.2	89.7 A	222.1 a	179.1 b	200.6 A	2.63	2.04	2.34 A
Hot humid (HH)	70.6	54.6	62.6 B	112.3 d	102.5 d	107.4C	1.59	1.90	1.75 B
Hot dry (HD)	66.1	81.3	73.7 AB	117.5 cd	135.5 c	126.5 B	1.78	1.67	1.72 B
Mean	73.9	76.7		150.6	139.0		2.00	1.87	
	$R_{d.25}$ (µmol CO_2 m^{-2} s^{-1})			$g_{m.25}$ (mol CO_2 m^{-2} s^{-1})			Γ (µmol mol^{-1})		
Normal (NC)	4.64	5.59	5.11 A	0.29	0.29	0.29 A	66.8	69.5	68.2C
Hot humid (HH)	2.03	1.86	1.94 B	0.05	0.036	0.04 B	93.4	99.7	96.5 A
Hot dry (HD)	1.20	1.15	1.18 B	0.06	0.06	0.06 B	83.6	80.2	81.9 B
Mean	2.62	2.86		0.136	0.133		81.3	83.2	
	A_{max} (µmol CO_2 m^{-2} s^{-1})			l_s (%)			CE (µmol CO_2 m^{-2} s^{-1})		
Normal (NC)	44.3 ab	35.5 c	39.9 B	38.3 ab	39.2 a	38.8 A	0.185 ab	0.187 ab	0.186 A
Hot humid (HH)	42.4 b	38.4 bc	40.4 B	31.4 bc	27.9 c	29.7 B	0.168 b	0.140 b	0.154 B
Hot dry (HD)	44.6 ab	50.9 a	47.8 A	35.3 ab	42.2 a	38.8 A	0.170 ab	0.219 a	0.195 A
Mean	43.8	41.6		35.0	36.4		0.175	0.182	

$V_{cmax.25}$, maximum rate of carboxylation by rubisco; $J_{.25}$, electron transport rate for RuBP regeneration; $R_{d.25}$, day respiration; $g_{m.25}$, mesophyll conductance; Γ, CO_2 compensation point; A_{max}, maximum net photosynthesis at 1500 PAR and 1500 ppm CO_2; l_s, stomatal limitation; CE, carboxylation efficiency of rubisco *in vivo*. $V_{cmax.25}$, $J_{.25}$, $R_{d.25}$ and $g_{m.25}$ parameters were normalized to 25 °C. Values sharing the same capital letter in a column, and in a row, do not differ significantly at P = 0.05 (Tukey HSD). Values sharing the same lower-case letter for climate-cultivar interaction, do not differ significantly at P = 0.05 (Tukey HSD).

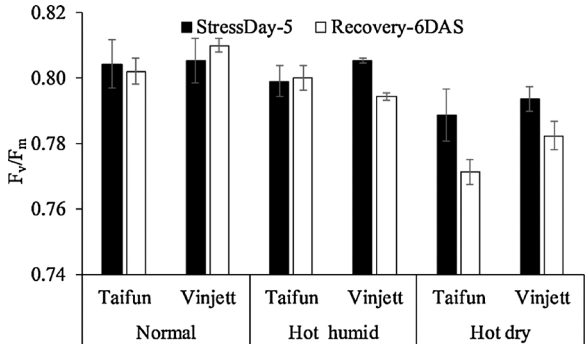

Fig. 4. Maximum efficiency of photosystem II of wheat cultivars (Taifun and Vinjett) as affected by climate (normal: 24 °C, 1.49 kPa VPD; hot humid: 36 °C, 1.96 kPa VPD; and hot dry: 36 °C, 3.92 kPa VPD) during the fifth day of stress period (black bars) and six days after the stress (white bars). Error bars represent ± SE (n = 3).

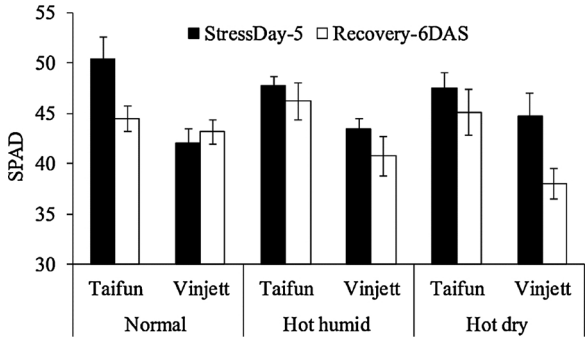

Fig. 5. Chlorophyll content index of wheat cultivars (Taifun and Vinjett) as affected by climate (normal: 24 °C, 1.49 kPa VPD; hot humid: 36 °C, 1.96 kPa VPD; and hot dry: 36 °C, 3.92 kPa VPD) during the fifth day of stress period (black bars) and six days after the stress (white bars). Error bars represent ± SE (n = 3).

Table 5
Parameters estimated from photosynthetic light-response curves after the stress period.

Climate	Taifun	Vinjett	Mean	Taifun	Vinjett	Mean
	AQY (mol mol^{-1})			A_{sat} (μmol CO_2 m^{-2}s^{-1})		
Normal (NC)	0.049	0.048	0.049	13.62	16.19	14.90
Hot humid (HH)	0.066	0.048	0.057	14.58	14.35	14.47
Hot dry (HD)	0.049	0.049	0.049	12.38	10.79	11.59
Mean	0.055	0.048		13.53	13.78	
	R_{dark} (μmol CO_2 m^{-2}s^{-1})			*LCP* (μmol CO_2 m^{-2}s^{-1})		
Normal (NC)	0.62	1.10	0.86	12.85	23.86	18.35
Hot humid (HH)	1.53	0.85	1.19	25.19	18.15	21.67
Hot dry (HD)	1.09	0.95	1.02	22.28	16.80	19.54
Mean	1.08	0.97		20.11	19.60	

AQY, apparent quantum yield; A_{sat}, maximum net photosynthesis at saturating light and 400 ppm CO_2; R_{dark}, dark respiration; LCP, light compensation point. Values sharing the same capital letter, in a column, and in a row, do not differ significantly at P = 0.05 (Tukey HSD). Values sharing the same lower-case letter for climate-cultivar interaction, do not differ significantly at P = 0.05 (Tukey HSD).

affected by climate, where HH resulted in significantly lower values than the NC and HD (Tables 2 and 6). The $J_{.25}$ was affected by climate × cultivar interaction where the Vinjett cultivar grown under NC had significantly higher $J_{.25}$ compared to Taifun under HD. The ratio of $J_{.25}$ to $V_{cmax.25}$ was significantly higher under HH than HD; however, the difference between HH and NC was not significant. A_{max} was also affected by climate × cultivar. A_{max} of Vinjett under NC was significantly higher from Taifun cultivar grown under HD climate. *In vivo* carboxylation efficiency estimated from *A-C$_i$* curves after the stress period was significantly lower under HH when compared to NC; however, statistically HH and HD were not different (Table 6).

3.2.3. Day respiration ($R_{d.25}$), mesophyll conductance ($g_{m.25}$), CO_2 compensation point (Γ) and relative stomatal limitation to photosynthesis (l_s)

Dark respiration ($R_{d.25}$), $g_{m.25}$ and Γ estimated from *A-Ci* curves measured after the stress period did not show any significant effect of climate, cultivar or climate × cultivar (Tables 2 and 6). Relative l_s was higher under NC than HH and HD climates (Table 6).

3.2.4. Maximum quantum efficiency of photosystem II (Fv/Fm) and chlorophyll content index (SPAD)

Maximum efficiency of PSII (F_v/F_m) was affected by climate. Plants under HD climate had significantly lower F_v/F_m than under NC and HH (Table 2; Fig. 4). SPAD was significantly higher for Taifun cultivar than Vinjett (Table 2; Fig. 5).

4. Discussion

Typically, photosynthesis is limited by maximum carboxylation rate of Rubisco *(V)* and electron transport rate for RuBP-regeneration *(J)* (Farquhar et al., 1980). The limiting step of photosynthesis is changed by (i) the balance between *J* and V_{cmax} and (ii) CO_2 concentration at the carboxylation site (Hikosaka et al., 2006). We observed that a heat-wave of five days significantly reduced the normalized values of $J_{.25}$, $J_{.25}/V_{cmax.25}$, $R_{d.25}$, $g_{m.25}$ and increased the chloroplast CO_2 compensation point, Γ (Table 4). Significant reduction in $J_{.25}/V_{cmax.25}$ suggested that CO_2 assimilation under HH and HD was mainly limited by RuBP regeneration which aligns with previous findings for wheat (Yamori et al., 2010). The decrease in $J_{.25}/V_{cmax.25}$ with temperature has partially been attributed to temperature induced changes in nitrogen partitioning in the photosynthetic apparatus, where the decrease in those proteins that are related to RuBP regeneration tends to be greater than decrease in Rubisco (Yamori et al., 2010). It is quite possible that the decrease in $J_{.25}/V_{cmax.25}$ with temperature in the present study was also due to the changes in photosynthetic enzymes because the PSII efficiency itself remained unaffected (Fig. 4) and cannot be held responsible for higher RuBP regeneration limitation (see below). Our results indicating a reduction in $J_{.25}/V_{cmax.25}$ with temperature contrasts to those of Bunce (2000), who reported that the balance between *J* and V_{cmax} is conserved, irrespective of growth temperature. However, in our study the balance and correlation between *J* and V_{cmax} were not conserved at different growth temperatures (Fig. 3).

Surprisingly, the maximum photosynthetic capacity under HH was reduced more than under HD (A_{max}; Table 4) which could be linked with RuBP regeneration limitation, lower actual carboxylation rate *in vivo (CE)* and lower $V_{cmax.25}$ (Table 4). It is known that the Rubisco enzyme is very stable at high temperature (Eckardt and Portis, 1997), and the carboxylation activity of fully active Rubisco increases with temperature within an optimum range (Salvucci and Crafts-Brandner, 2004), which varies between 35 and 41 °C for *in vivo* carboxylation activity (Bernacchi et al., 2001; Medlyn et al., 2002). Regardless of cultivars and VPD, we also observed temperature induced increase in V_{cmax}; however, this increase was higher under HD than under HH (Supplementary material Table 1S). Relatively lower increase in V_{cmax} and lower *CE* and $V_{cmax.25}$ values under HH can be ascribed to decline in Rubisco activation, while the higher increase in V_{cmax} under HD indicated the maintenance of relatively high Rubisco activation state. The decline in Rubisco activation is highly possible because Rubisco activase is very sensitive to high temperature (Law and Crafts-Brandner, 1999; Salvucci and Crafts-Brandner, 2004; Salvucci et al., 2001) and drought (Parry et al., 2002). The increase in V_{cmax} with temperature was also reported by Stasik and Jones (2007), who associated it with a relatively higher Rubisco activation state in heat tolerant compared to heat sensitive wheat cultivars.

The HH climate being more stressful than HD was unexpected, and against our primary hypothesis that HD will be more severe due to both direct (heat stress) and indirect (high VPD driven drought stress) effects

Table 6
Parameters estimated from photosynthetic CO_2 response curves after the stress period.

Climate	Taifun	Vinjett	Mean	Taifun	Vinjett	Mean	Taifun	Vinjett	Mean
	$V_{cmax.25}$ (μmol CO_2 $m^{-2}s^{-1}$)			$J_{.25}$ (μmol e^- $m^{-2}s^{-1}$)			$J_{.25}/V_{cmax.25}$		
Normal (NC)	83.0	104.0	93.5 A	150.2 ab	192.2 a	171.2	1.94	1.93	1.93 AB
Hot humid (HH)	66.3	55.7	61.0 B	160.7 ab	145.4 ab	153.1	2.42	2.63	2.53 A
Hot dry (HD)	96.6	88.4	92.5 A	138.2 b	160.6 ab	149.4	1.49	1.87	1.68 B
Mean	82.0	82.7		149.7	166.1		1.95	2.14	
	$R_{d.25}$ (μmol CO_2 $m^{-2}s^{-1}$)			$g_{m.25}$ (mol CO_2 $m^{-2}s^{-1}$)			Γ (μmol mol^{-1})		
Normal (NC)	4.93	5.71	5.32	0.24	0.35	0.29	69.1	67.3	68.2
Hot humid (HH)	4.15	4.60	4.38	0.33	0.23	0.28	71.5	81.4	76.5
Hot dry (HD)	6.31	5.88	6.09	0.46	0.32	0.39	72.2	73.6	72.9
Mean	5.13	5.40		0.34	0.30		70.9	74.1	
	A_{max} (μmol CO_2 $m^{-2}s^{-1}$)			l_s (%)			CE (μmol CO_2 $m^{-2}s^{-1}$)		
Normal (NC)	29.9 ab	39.3 a	34.1	35.0	37.9	36.4 A	0.160	0.164	0.162 A
Hot humid (HH)	32.5 ab	28.8 ab	30.6	28.7	27.3	28.0 B	0.135	0.122	0.128 B
Hot dry (HD)	27.3 b	32.0 ab	29.7	29.8	28.9	29.3 B	0.148	0.157	0.153 AB
Mean	29.9	33.0		31.2	31.4		0.148	0.148	

$V_{cmax.25}$, maximum rate of carboxylation by rubisco; $J_{.25}$, electron transport rate for RuBP regeneration; $R_{d.25}$, day respiration; $g_{m.25}$, mesophyll conductance; Γ, CO_2 compensation point; A_{max}, maximum net photosynthesis at 1500 PAR and 1500 ppm CO_2; l_s, stomatal limitation; CE, carboxylation efficiency of rubisco *in vivo*. $V_{cmax.25}$, $J_{.25}$, $R_{d.25}$ and $g_{m.25}$ parameters were normalized to 25 °C. Values sharing the same capital letter in a column, and in a row, do not differ significantly at P = 0.05 (Tukey HSD). Values sharing the same lower-case letter for climate-cultivar interaction, do not differ significantly at P = 0.05 (Tukey HSD).

of high temperature. Moreover, as the temperature-induced increase in VPD reduces g_s (Kröber and Bruelheide, 2014; Kröber et al., 2015), the net photosynthesis could be limited by lower C_i. However, despite having high g_s (Supplementary material Fig. 1S), $V_{cmax.25}$, *CE* and A_{max} were relatively lower (though not always significant) and Γ was higher under HH compared to HD climate (Table 4). This could not be explained by differences in respiratory losses ($R_{d.25}$) and mesophyll conductance ($g_{m.25}$), both of which were not statistically different for the two hot climates (Table 4). The major reason behind this discrepancy was that the HH turned out to be more severe than HD. This was because of the increase in g_s and TR over time due to acclimation (Rashid et al., 2018). Higher g_s under HH increased TR and the difference in TR between HH and HD was reduced (Supplementary material Fig. 2S). As a relatively lower amount of water was applied to HH than to HD (see Materials and methods); increased TR might have invoked drought stress under HH, ultimately making it more severe than HD. The water application under different climates was based on initial estimates of evapotranspiration but as the stress-period progressed, plants acclimatized to the new environment including an increase in g_s and TR under HH (Rashid et al., 2018). Higher g_s under high temperature has been suggested as an advantage for plants due to evaporative cooling (Fischer et al., 1998; Gourdji et al., 2013); however, higher transpiration could also lead to drought stress under water limited conditions. The induced drought, however, did not lower the g_s and C_i in present study. This could partially be explained by the fact that plants growing under high humidity (*i.e.* HH) lose control on the stomatal functioning and stomata does not respond to the environmental and/or hormonal stimuli such as light, CO_2, desiccation/ABA (Aliniaeifard and van Meeteren, 2013). We also observed that the stomata under HH conditions tended to be less responsive to changes in light and CO_2 (Supplementary material Figs. 1S and 4S ; referring to the g_s at lower light and higher CO_2 levels comparing HH and HD during the stress).

Nonetheless, the effect of HD on maximum photosynthetic capacity was not as severe as we hypothesised, and in fact it was less severe than of HH. The higher g_s under HH did not result in higher A_{max}, and the lower g_s under HD did not significantly contribute to decrease in A_{max}. Therefore, higher stomatal limitation (l_s) under HD was dwarfed by higher non-stomatal limitation (*CE*, $J_{.25}$, $V_{cmax.25}$) under HH due to combined effect of temperature and induced drought (Table 4). The lack of significant effect of lower g_s under HD on *CE*, $V_{cmax.25}$ *and* A_{max} may be attributed to the stable nature of C_i, since it has been reported that despite changes in g_s, C_i may remain constant for a fixed external CO_2 concentration, and the g_s itself tends to have little effect on photosynthesis unless it drops to quite low values (Bunce, 2000). Other reports (Law and Crafts-Brandner, 1999; Stasik and Jones, 2007) have also reported non-significant effect of g_s on photosynthetic capacity in heat stress experiments. Nonetheless, despite having relatively lower g_s and thus higher stomatal limitation (l_s), the photosynthetic capacity (A_{max}) under HD was not reduced (Table 4), because the C_i remained fairly stable across climates (Supplementary material Fig. 3S). This was probably because the reduced g_s was not too low to limit CO_2 diffusion. Moreover, the CO_2 diffusion through stomata tends to be faster at higher temperatures. Hence, the non-stomatal limitation prevailed both under HH and HD. Changes in A_{max} under high temperature (Table 4) can very well be explained by changes in V_c, J, Γ and R_d (Supplementary material Table 1S). Conversely, the response of A_{max} could not be attributed to changes in g_m. As g_m decreased at high temperature, it should have negatively influenced the A_{max}, which was not the case (Table 4). This is probably because the contribution of g_m in changing the CO_2 concentration from intercellular spaces to carboxylation site (chloroplast) in wheat is small and will not influence photosynthesis at moderate high temperature *i.e.* < 40 °C (Perdomo et al., 2017).

The analysis of photosynthetic response curves indicated that heat stress affected the photosynthetic capacity mainly through effects on biochemical processes that are involved in CO_2-fixation, while those of light-use remained largely unaffected. Although, the *AQY* and *LCP* were respectively lower and higher under high temperature treatments, A_{sat} remained unaffected (Table 3). This was also the case for the two cultivars, which regardless of having different *AQY* did not differ for highest operational net photosynthetic rate (A_{sat}). Although, the maximum efficiency of PSII (F_v/F_m) was relatively lower under HD (Fig. 4), it did not influence the net assimilation rate. This could be attributed to the fact that the values of F_v/F_m were always higher than 0.78 (Fig. 4), which is the lower limit of non-stressed C_3 plants (Maxwell and Johnson, 2000). Our results confirm the findings of Law and Crafts-Brandner (1999), who reported that the Calvin cycle is more sensitive to high temperature than PSII activity. Law and Crafts-Brandner (1999) also reported the acclimation potential of PSII in wheat. More recently, Sharkey (2005) and Stasik and Jones (2007) showed that the PSII activity remains unaffected even at temperatures high enough to inhibit CO_2 fixation. Despite the lack of any significant inhibition of PSII activity (Fig. 4), RuBP regeneration limitation to photosynthesis was higher under high temperature (HH and HD) (Table 4). This could partially be linked with enhanced activity of alternative electron sinks (Stasik and Jones, 2007). Nonetheless, potentially insignificant effect of temperature on PSII efficiency (Fig. 4), chlorophyll content index

(SPAD) (Fig. 5) and the highest net assimilation rate (A_{sat}) (Table 3) supported our judgement that heat stress influenced photosynthesis largely by affecting the processes that are involved in Calvin cycle, *i.e.* *CE* and V_{cmax}.

Analysis of *A*-C_i and *A*-*Q* curves measured after six days of recovery indicated that most of the photosynthetic and related parameters were not significantly different from their respective control, and this was irrespective of the stress treatment (Tables 2, 5 and 6). The plants which had been under HH treatment still had relatively lower *CE*, V_{cmax} and higher J/V_{cmax} indicating higher RuBP carboxylation limitation; however, the maximum photosynthetic capacity remained unaffected; A_{max} (Table 6). The same was true for the plants under HD which despite having lower J/V_{cmax} (Table 6) and lower F_v/F_m (Fig. 4) had similar Nevertheless, the lasting-effects of high temperature and VPD on photosynthetic capacity when measured after six days under normal climate were either non-existent or potentially insignificant. These results suggest that the effects of moderately high temperature (36 °C) with high and low VPD were time-limited with no significant damage to photosynthetic apparatus. Our results confirm that the photosynthetic apparatus (Rubisco and PSII efficiency) itself is quite stable at moderately high temperature. It is known that the damage to photosynthetic apparatus is done at extreme high temperatures such as > 41 °C for Rubisco (Bernacchi et al., 2001; Medlyn et al., 2002) and > 45 °C for PSII (Sharkey, 2005). Thus, the moderate high temperature such as in present study can affect the operating efficiency, but may not cause permanent damage.

5. Conclusions

Regardless of VPD, photosynthesis under high temperature was primarily limited by RuBP regeneration. Photosynthesis under high temperature and low VPD was co-limited by RuBP carboxylation and RuBP regeneration. This was because significantly higher g_s and increase in g_s and transpiration over time (acclimation) led to drought, thus intensifying the biochemical limitation to photosynthesis. Photosynthesis under high temperature was largely impaired by biochemical limitations while the diffusional limitations were potentially insignificant. From the biochemical limitations, the processes related to CO_2-use (carboxylation efficiency, V_{cmax}) were more sensitive than the processes involved in light-use (PSII efficiency, quantum yield, SPAD). The influence of high and low VPD on photosynthesis through changes in stomatal conductance and intercellular CO_2 concentration was small. Both cultivars responded similarly to climate treatments for most of the photosynthetic parameters. The effects of high temperature and VPD were short-lived, and most of the photosynthetic- and related parameters recovered after the stress.

Conflicts of interest

None.

Acknowledgements

Financial support for this work was provided by the Sino-Danish Center for Education and Research. Additional funding was provided by the Graduate School of Science and Technology, Aarhus University. Technical support from Helle Baadsgaard Sørensen is highly appreciated and acknowledged.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.envexpbot.2017.12.009.

References

Ainsworth, E.A., Ort, D.R., 2010. How do we improve crop production in a warming world? Plant Physiol. 154, 526–530.
Aliniaeifard, S., van Meeteren, U., 2013. Can prolonged exposure to low VPD disturb the ABA signalling in stomatal guard cells? J. Exp. Bot. 64, 3551–3566.
Allakhverdiev, S.I., Kreslavski, V.D., Klimov, V.V., Los, D.A., Carpentier, R., Mohanty, P., 2008. Heat stress: an overview of molecular responses in photosynthesis. Photosynth. Res. 98, 541.
Asseng, S., Foster, I.A.N., Turner, N.C., 2011. The impact of temperature variability on wheat yields. Glob. Change Biol. 17, 997–1012.
Asseng, S., Ewert, F., Martre, P., et al., 2015. Rising temperatures reduce global wheat production. Nat. Clim. Change 5, 143–147.
Atkin, O.K., Scheurwater, I., Pons, T.L., 2006. High thermal acclimation potential of both photosynthesis and respiration in two lowland Plantago species in contrast to an alpine congeneric. Glob. Change Biol. 12, 500–515.
Bernacchi, C.J., Singsaas, E.L., Pimentel, C., Portis Jr., A.R., Long, S.P., 2001. Improved temperature response functions for models of Rubisco-limited photosynthesis. Plant Cell Environ. 24, 253–259.
Bernacchi, C.J., Pimentel, C., Long, S.P., 2003. In vivo temperature response functions of parameters required to model RuBP-limited photosynthesis. Plant Cell Environ. 26, 1419–1430.
Bunce, J.A., 2000. Acclimation of photosynthesis to temperature in eight cool and warm climate herbaceous C3 species: temperature dependence of parameters of a biochemical photosynthesis model. Photosynth. Res. 63, 59–67.
Crafts-Brandner, S.J., Salvucci, M.E., 2000. Rubisco activase constrains the photosynthetic potential of leaves at high temperature and CO2. Proc. Natl. Acad. Sci. 97, 13430–13435.
Eckardt, N.A., Portis Jr., A.R., 1997. Heat denaturation profiles of ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) and Rubisco activase and the inability of Rubisco activase to restore activity of heat-denatured Rubisco. Plant Physiol. 113, 243–248.
Ethier, G.J., Livingston, N.J., 2004. On the need to incorporate sensitivity to CO2 transfer conductance into the Farquhar–von Caemmerer–Berry leaf photosynthesis model. Plant Cell Environ. 27, 137–153.
Farooq, M., Bramley, H., Palta, J.A., Siddique, K.H.M., 2011. Heat stress in wheat during reproductive and grain-filling phases. Crit. Rev. Plant Sci. 30, 491–507.
Farquhar, G.D., Sharkey, T.D., 1982. Stomatal conductance and photosynthesis. Annu. Rev. Plant Physiol. 33, 317–345.
Farquhar, G.D., von Caemmerer, S., Berry, J.A., 1980. A biochemical model of photosynthetic CO2 assimilation in leaves of C3 species. Planta 149, 78–90.
Feller, U., Crafts-Brandner, S.J., Salvucci, M.E., 1998. Moderately high temperatures inhibit ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) activase-mediated activation of Rubisco. Plant Physiol. 116, 539–546.
Ferris, R., Ellis, R.H., Wheeler, T.R., Hadley, P., 1998. Effect of high temperature stress at anthesis on grain yield and biomass of field-grown crops of wheat. Ann. Bot. 82, 631–639.
Fischer, R.A., Rees, D., Sayre, K.D., Lu, Z.M., Condon, A.G., Saavedra, A.L., 1998. Wheat yield progress associated with higher stomatal conductance and photosynthetic rate and cooler canopies. Crop Sci. 38, 1467–1475.
Genty, B., Briantais, J.-M., Baker, N.R., 1989. The relationship between the quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence. Biochim. Biophys. Acta (BBA)–Gen. Subj. 990, 87–92.
Gourdji, S.M., Mathews, K.L., Reynolds, M., Crossa, J., Lobell, D.B., 2013. An assessment of wheat yield sensitivity and breeding gains in hot environments. Proc. R. Soc. Lond. B: Biol. Sci. 280.
Harley, P.C., Thomas, R.B., Reynolds, J.F., Strain, B.R., 1992. Modelling photosynthesis of cotton grown in elevated CO2. Plant Cell Environ. 15, 271–282.
Hikosaka, K., Ishikawa, K., Borjigidai, A., Muller, O., Onoda, Y., 2006. Temperature acclimation of photosynthesis: mechanisms involved in the changes in temperature dependence of photosynthetic rate. J. Exp. Bot. 57, 291–302.
IPCC, 2012. Managing the Risks of Extreme Events and Disasters to Advance Climate Change Adaptation. A Special Report of Working Groups I and II of the Intergovernmental Panel on Climate Change. IPCC Cambridge uni. press, Cambridge UK, and New York, NY, USA.
IPCC, 2013. Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change.
Kröber, W., Bruelheide, H., 2014. Transpiration and stomatal control: a cross-species study of leaf traits in 39 evergreen and deciduous broadleaved subtropical tree species. Trees 28, 901–914.
Kröber, W., Plath, I., Heklau, H., Bruelheide, H., 2015. Relating stomatal conductance to leaf functional traits. J. Vis. Exp.: JoVE 52738.
Lancashire, P.D., Bleiholder, H., Boom, T.V.D., LangelÜDdeke, P., Stauss, R., Weber, E., Witzenberger, A., 1991. A uniform decimal code for growth stages of crops and weeds. Ann. Appl. Biol. 119, 561–601.
Law, R.D., Crafts-Brandner, S.J., 1999. Inhibition and acclimation of photosynthesis to heat stress is closely correlated with activation of ribulose-1,5-bisphosphate carboxylase/oxygenase. Plant Physiol. 120, 173–182.
Marshall, B., Biscoe, P.V., 1980. A model for C3 leaves describing the dependence of net photosynthesis on irradiance. J. Exp. Bot. 31, 29–39.
Maxwell, K., Johnson, G.N., 2000. Chlorophyll fluorescence—a practical guide. J. Exp. Bot. 51, 659–668.
Medlyn, B.E., Dreyer, E., Ellsworth, D., Forstreuter, M., Harley, P.C., Kirschbaum, M.U.F., Le Roux, X., Montpied, P., Strassemeyer, J., Walcroft, A., Wang, K., Loustau, D., 2002.

Temperature response of parameters of a biochemically based model of photosynthesis. II. A review of experimental data. Plant Cell Environ. 25, 1167–1179.
Parry, M.A.J., Andralojc, P.J., Khan, S., Lea, P.J., Keys, A.J., 2002. Rubisco activity: effects of drought stress. Ann. Bot. 89, 833–839.
Parsons, R., Weyers, J.D.B., Lawson, T., Godber, I.M., 1998. Rapid and straightforward estimates of photosynthetic characteristics using a portable gas exchange system. Photosynthetica 34, 265–279.
Perdomo, J.A., Capó-Bauçà, S., Carmo-Silva, E., Galmés, J., 2017. Rubisco and Rubisco activase play an important role in the biochemical limitations of photosynthesis in rice, wheat, and maize under high temperature and water deficit. Front. Plant Sci. 8, 490.
Perez-Martin, A., Flexas, J., Ribas-Carbó, M., Bota, J., Tomás, M., Infante, J.M., Diaz-Espejo, A., 2009. Interactive effects of soil water deficit and air vapour pressure deficit on mesophyll conductance to CO2 in Vitis vinifera and Olea europaea. J. Exp. Bot. 60, 2391–2405.
Porter, J.R., Gawith, M., 1999. Temperatures and the growth and development of wheat: a review. Eur. J. Agron. 10, 23–36.
Rashid, M.A., Andersen, M.N., Wollenweber, B., Zhang, X., Olesen, J.E., 2018. Acclimation to higher VPD and temperature minimized negative effects on assimilation and grain yield of wheat. Agric. For. Meteorol. 248, 119–129.
Sage, R.F., Way, D.A., Kubien, D.S., 2008. Rubisco, Rubisco activase, and global climate change. J. Exp. Bot. 59, 1581–1595.
Salvucci, M.E., Crafts-Brandner, S.J., 2004. Inhibition of photosynthesis by heat stress: the activation state of Rubisco as a limiting factor in photosynthesis. Physiol. Plant. 120, 179–186.
Salvucci, M.E., Osteryoung, K.W., Crafts-Brandner, S.J., Vierling, E., 2001. Exceptional sensitivity of Rubisco activase to thermal denaturation in vitro and in vivo. Plant Physiol. 127, 1053–1064.
Shapiro, S.S., Wilk, M.B., 1965. An analysis of variance test for normality (complete samples). Biometrika 52, 591–611.
Sharkey, T.D., Bernacchi, C.J., Farquhar, G.D., Singsaas, E.L., 2007. Fitting photosynthetic carbon dioxide response curves for C3 leaves. Plant Cell Environ. 30, 1035–1040.
Sharkey, T.D., 2005. Effects of moderate heat stress on photosynthesis: importance of thylakoid reactions, rubisco deactivation, reactive oxygen species, and thermotolerance provided by isoprene. Plant Cell Environ. 28, 269–277.
Stasik, O., Jones, H.G., 2007. Response of photosynthetic apparatus to moderate high temperature in contrasting wheat cultivars at different oxygen concentrations. J. Exp. Bot. 58, 2133–2143.
Trnka, M., Rotter, R.P., Ruiz-Ramos, M., Kersebaum, K.C., Olesen, J.E., Zalud, Z., Semenov, M.A., 2014. Adverse weather conditions for European wheat production will become more frequent with climate change. Nat. Clim. Change 4, 637–643.
Vignjevic, M., Wang, X., Olesen, J.E., Wollenweber, B., 2015. Traits in spring wheat cultivars associated with yield loss caused by a heat stress episode after anthesis. J. Agron. Crop Sci. 201, 32–48.
Wang, X., Dinler, B.S., Vignjevic, M., Jacobsen, S., Wollenweber, B., 2015. Physiological and proteome studies of responses to heat stress during grain filling in contrasting wheat cultivars. Plant Sci. 230, 33–50.
Will, R.E., Wilson, S.M., Zou, C.B., Hennessey, T.C., 2013. Increased vapor pressure deficit due to higher temperature leads to greater transpiration and faster mortality during drought for tree seedlings common to the forest–grassland ecotone. New Phytol. 200, 366–374.
Wollenweber, B., Porter, J.R., Schellberg, J., 2003. Lack of interaction between extreme high-temperature events at vegetative and reproductive growth stages in wheat. J. Agron. Crop Sci. 189, 142–150.
Yamane, Y., Kashino, Y., Koike, H., Satoh, K., 1998. Effects of high temperatures on the photosynthetic systems in spinach oxygen-evolving activities, fluorescence characteristics and the denaturation process. Photosynth. Res. 57, 51–59.
Yamori, W., Noguchi, K., Hikosaka, K., Terashima, I., 2010. Phenotypic plasticity in photosynthetic temperature acclimation among crop species with different cold tolerances. Plant Physiol. 152, 388–399.
Zhang, R., Sharkey, T.D., 2009. Photosynthetic electron transport and proton flux under moderate heat stress. Photosynth. Res. 100, 29–43.